

# LC/MS DETERMINATION OF AMINO ACIDS USING COMPACT MASS SPECTROMETRY

## **APPLICATION NOTE**

**Mass Spec:** expression CMS  
**LC System:** UHPLC

In this application note, we show examples for the determination of amino acids on a combined HPLC compact mass spectrometry (LC/CMS) system using the Advion expression CMS for the rapid detection and quantification of amino acids utilizing a novel mixed mode LC column.

*Advion, Inc.*

## INTRODUCTION

Amino acids are the building blocks of the protein backbone and universally contain an amine and carboxylic acid functional group. Free amino acid blood determination is important for the early detection of inborn errors of metabolism in the newborn screening program. Also, amino acid usage has increased in the food industry and requires product control analysis.

HPLC is the most common separation technique for the analysis of samples containing amino acids, with traditional approaches using pre- or post-column derivatization and UV detection. However, derivatization adds unnecessary sample preparation steps, and using mass spectrometry as the detector allows for overlapping analyte peaks and hence, faster HPLC analysis times.

In this application note, we show examples for the determination of amino acids on a combined HPLC compact mass spectrometry (LC/CMS) system with the Advion **expression** CMS for the rapid detection and quantification of amino acids utilizing a novel mixed mode LC column. This sample analysis strategy results in a cost effective analysis system to detect and quantify free amino acids in mixtures, protein amino acid compositions and amino acid biomarkers.

## METHOD

### LC Method

Column: 150 mm x 2 mm Intrada with 3  $\mu$ m particles (Imtakt Corporation, Japan)

Flow Rate: 200–400  $\mu$ L/min.

Solvent A: Acetonitrile/Tetrahydrofuran/25 mM Ammonium formate/Formic Acid 9/75/16/0.3 (v:v:v:v)

Solvent B: Acetonitrile/100 mM Ammonium formate 20/80 (v:v).

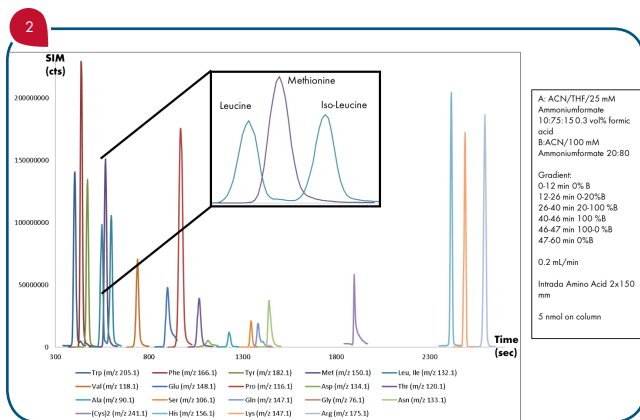


**Figure 1:** UHPLC/CMS set up for the detection and quantification of amino acids in complex mixtures utilizing a JASCO X-LC system, an Imtakt Corporation Intrada Amino Acid HPLC column, and the Advion **expression**<sup>+</sup> CMS.

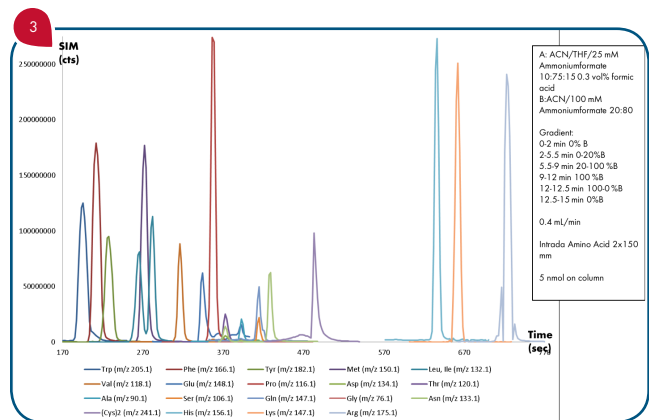
## RESULTS

A first separation example is shown in Figure 2 with a 60 min chromatography separation and LC/CMS detection of a standard 20 amino acid mixture using selected ion monitoring (SIM) acquisition for each analyte. Here, a complete baseline separation can be achieved and isotopic analytes such as Leucine and Iso-Leucine are baseline separated and readily distinguishable by their unique retention times.

However, due to the selectivity of the CMS detector, a baseline separation of all analytes is not necessarily required and faster chromatography can be used to analyze the 20 amino acid mixture in only 15 min (Figure 3) – a significant increase in sampling speed. Now, the Leu/Ile pair is still resolved, albeit not baseline separated, and some other analytes are co-eluting. But since the co-eluting peaks have different mass-to-charge ( $m/z$ ) ratios, they can easily be distinguished by mass spectrometry, something not possible in UV detection with or without derivatization. Figure 4 shows example calibration functions for four amino acids (Proline, Tyrosine, Phenylalanine, and Threonine).

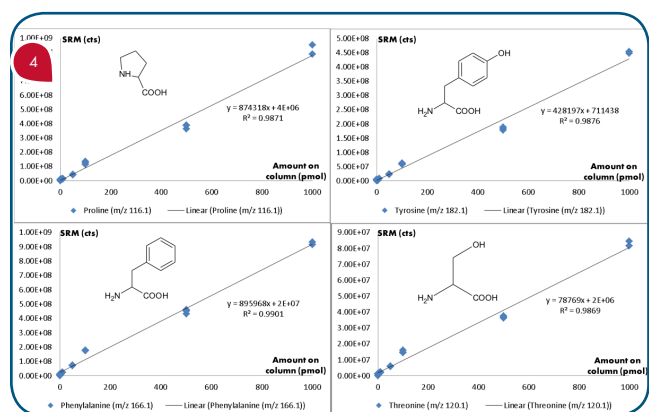


**Figure 2:** Example of a 60 min LC/CMS analysis of a 20 amino acid mixture using SIM (5 nmol on column).



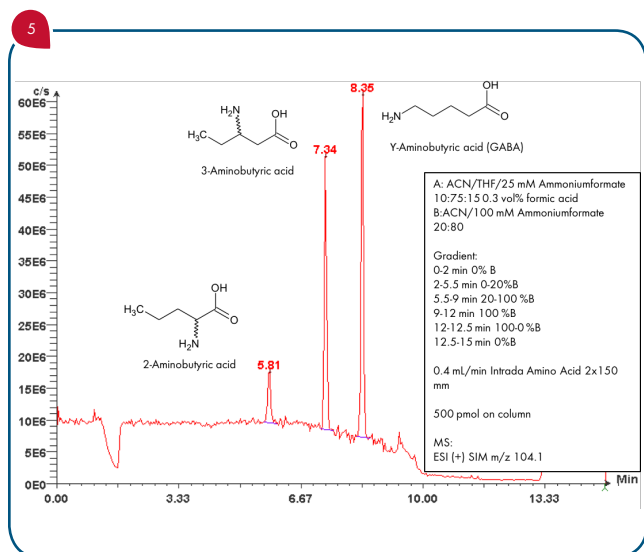
**Figure 3:** Example of a 15 min LC/CMS analysis of a 20 amino acid mixture using SIM acquisition of the unique  $m/z$  of each amino acid.

Tyrosine, Phenylalanine and Threonine) with good linearity in the range of 5 to 1000 pmol on-column (ca. 600 pg to 180 ng of the respective amino acid). This analysis employed the 15 min chromatography for a faster sample throughput. Observed linearity and sensitivity is well within the required levels for eg. amino acid blood determination.

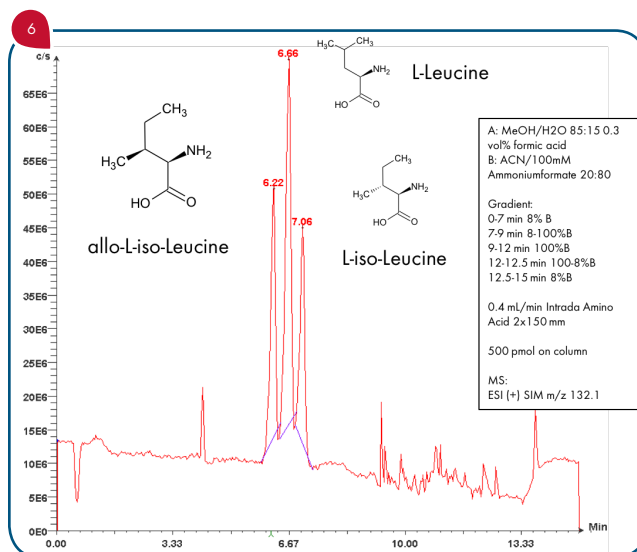


**Figure 4:** Calibration functions for Proline, Tyrosine, Phenylalanine, and Threonine.

To further show the potential of the LC/CMS analysis system, Figure 5 shows the separation and detection gamma-aminobutyric acid (GABA) isomers. All three isotopic and isobaric analytes are baseline-separated and can be readily distinguished. The same can be shown for a mixture of isotopes of Leucine (Figure 6). Here, the mobile phase solvents had to be slightly adjusted and gradient conditions changed as indicated to achieve a separation of all three isotopes.



**Figure 5:** Separation of GABA isomers using the same 15 min LC/CMS method than before



**Figure 6:** Separation of L-Leucine isomers using a modified 15 min gradient.

## CONCLUSIONS

A combination of ion-ion interaction chromatography and a CMS presents a cost-effective and powerful analysis system for the detection and quantification of amino acids in complex mixtures without the need for extra sample processing steps such as derivatizations.

### ACKNOWLEDGEMENT

We would like to thank Itaru Yazawa from Imtakt Corporation, Japan for the generous donation of an Intrada Amino Acid HPLC column and JASCO Corporation, USA for the loan of a UHPLC system.